# Nitrate reductase activity and its diurnal variation rhythm for *Camptotheca acuminata* seedlings

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**Abstract:** Nitrate reductase activity ( NRA ) in different plant organs and leaves in different positions of *Camptotheca acuminata* seedlings was determined by an *In vivo* assay, the diurnal variation rhythm of NRA in leaves of different positions was observed, and the correlations between leaf NRA, leaf area and lamina mass per unit area (LMA) were also examined. The results showed that NRA in the leaf was significantly highest, compared with that in other organs such as roots, stems and leaves. In this experiment, the 10 leaves were selected from the apex to the base of the seedlings in order. The different NRA occurred obviously in leaves of different positions of *C. acuminata* seedlings from the apex to the base, and NRA was higher in the 4th-6th leaves. The diurnal change rhythm of leaf NRA showed a one peak curve, and maximum NRA value appeared at about midday (at 12:30 or so). No obvious correlations between NRA and leaf area or lamina mass per unit area were observed. This study offered scientific foundation for the further research on nitrogen metabolism of *C. acuminata*.

**Keywords:** *Camptotheca acuminata;* Nitrate reductase activity; Diurnal variation
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## Introduction

Nitrate ($NO_3^-$) is the primary source of nitrogen for most land plants, and it also acts as a signal, which stimulates the coordinated change of carbon and nitrogen metabolism of plants (Scheible *et al.*1997). Nitrate reductase (NR) is the first key enzyme of $NO_3^-$ assimilation and it can regulate the plant nitrogen nutrition and has an important effect on photosynthesis, respiration and carbon metabolism, etc. (Vogel *et al.* 1991). Nitrate reductase activity (NRA) may explain the differences in response to nitrogen from different plants, its variation results from the interaction between plants and nitrogen nutrition under some environmental conditions (Black *et al.* 2002; Castle *et al.* 2003). Some recent works have revealed that NRA had variations in different plant organs and developmental stages (Wang *et al.* 2003; Castle *et al.* 2003), and its diurnal variation rhythm was also found in leaves, shoots and roots of a number of higher plants (Wen *et al.* 1999; Söthr *et al.* 2001; Aslam *et al.* 2001). However, the research on NRA in leaves of different positions and organs of *C. acuminata* seedlings has rarely been reported. In this study, NRA in leaves of different positions and different organs of *C. acuminata* seedlings was assayed, its diurnal variation rhythm in leaves was observed, and the correlations between leaf NRA and leaf area and lamina mass per unit area (LMA) were also examined. This study may provide some information for the further research on nitrogen metabolism of *C. acuminata*.

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## Materials and methods

### Plant materials

Seedlings with six leaves in a greenhouse were transplanted to the outdoors ground at the end of May, 2003, and no supplemental fertilizers were supplied. When seedlings with 12-15 leaves were about 35-40 cm height, they were selected for experimental materials. In this experiment, a leaf which has obviously distinguishable shoot and node was defined as leaf 1, the following was as leaf 2, leaf 3, leaf 4....., and leaf 10 at last from the apex to the base for the same seedling. The shoot located between leaf 1 and leaf 2 was assigned the first internode. Each leaf was assayed as one sample. Leaf 3 to leaf 10 were picked up from five uniform seedlings (5 replicates) to assay NRA in leaves of different positions.

### NRA in leaves of different position

NRA in plants can be improved by $KNO_3$ solution (Zhou *et al.* 1998). Thus, the intact seedlings were placed in the 50 mmol/L-$KNO_3$ solution and induced for 24 h to determine the NRA in leaves of different positions for each seedling.

### NRA in various organs

Ten seedlings are evenly separated into two groups. One was treated with 50 mmol/L-$KNO_3$ solution, and other was treated as the control. Each seedling was separated into four parts: roots (rootlet, diameter<2 mm), shoots (phloem located between the 1st internode and the 5th internode), leaves (leaf 4 to leaf 6) and shoot tips (including the first

unexpanded young leaf).

**Diurnal variation rhythm of NRA**

On August 30. 2003, leaf 4 to leaf 6 were sampled from four uniform seedlings at 7:30, 9:30, 10:30, 11:30, 12:30, 13:15, 14:00, 15:30, 18:00 hours respectively (4 replicates) and the NRA of leaf 4 to leaf 6 from the same seedling was assayed as one sample.

**Relationship between leaf NRA and leaf characteristics**

Five leaves with different areas were randomly selected from each of five uniform seedlings. After leaf area of each leaf was measured by a LI-3000A Portable Area Meter (LI-COR Co., USA), the leaf NRA was assayed and LMA of each leaf was calculated as one sample. NRA was determined by a modified *in vivo* assay as described by Sun and Yan (2004).

## Results and discussion

**NRA variation in organs of *C. acuminata* seedlings**

NRA in leaves, whether seedlings were induced by $KNO_3$-solution or not, was significantly higher than that in the other organs (Fig.1), NRA in shoots and in shoot tips was relatively lower, but had no significant difference. No NRA in roots was detected under the conditions of our experiment. In addition, NRA increased 54.7%, 81.7% and 73.5% in leaves, shoots and shoot tips respectively after being induced by $KNO_3$, compared with that in the control.

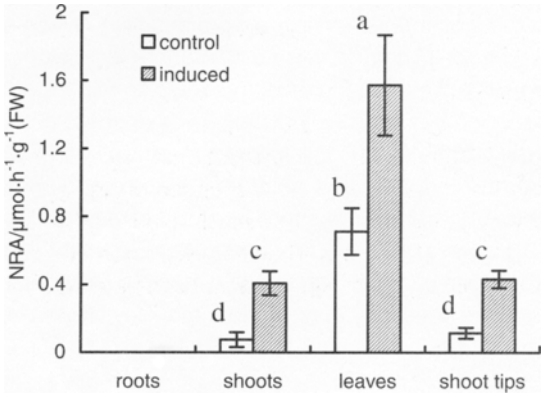


**Fig.1 Comparison of NRA in different organs of *C. acuminata* seedlings**

Values represent the means ± SE (n=5), different letters indicate significant differences between the means ($P$<0.05)

The results from previous studies ((Lambers *et al.* 1998; Högberg *et al.* 1998) were not consistent with our experimental results. Lambers (1998) reported that the highest NRA presented in roots of some plant species, due to different plant species and their habitat. The relative importance of various plant tissues for $NO_3^-$ assimilation has usually been assessed on the basis of the proportions of NRA in various tissues. The pattern of $NO_3^-$ assimilation and partitioning, etc. may be analyzed at whole plant level (Aslam *et al.* 2001). However, in our experiment, the NRA in leaves was significantly much higher than that in the other organs of *C. acuminate*; it may suggest that leaves are the major tissues of $NO_3^-$ reduction and assimilation.

**NRA in leaves of different positions**

NRA was distinctly different in leaves of different positions (Fig. 2). From the apex of seedling, NRA in leaf 3 was relatively lower, whereas the relatively higher NRA was found between leaf 4 and leaf 6 (leaf 6 is the largest one for the same seedling), and the maximum NRA was 2.38 $\mu mol \cdot h^{-1} \cdot g^{-1}$ (FW). Thereafter, NRA almost declined linearly from leaf 6 to leaf 10, and the lowest NRA was only 0.66 $\mu mol \cdot h^{-1} \cdot g^{-1}$ (FW) in leaf 10.

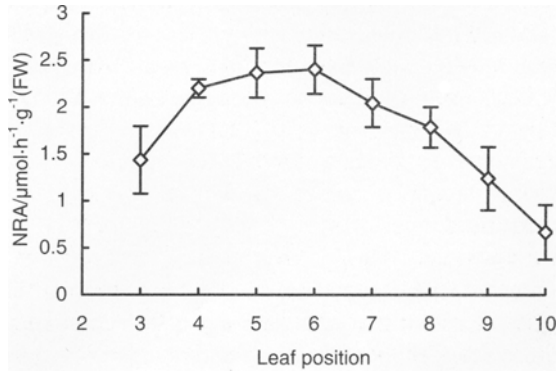


**Fig. 2 Changes of NRA in leaves of different positions of *C. acuminata* seedlings**

Some results suggested that leaf NRA changes displayed various patterns in different plant species. NRA in leaf of tobacco and peach seedlings declines in different position leaves from the apex to the base. Jin *et al.* (2003) also found that leaf NRA in pear seedlings tended to increase from the base to the apex. On the other hand, in some researches, NRA in leaves has been also examined from the point of view of leaf age. Hunter and Ruffner (1997) pointed out that NRA was lower in young leaves than that in mature leaves of grapevine. Namely, there is relatively higher NRA in mature leaves than in young ones. Still Black *et al.* (2002) reported that the decrease of NRA with increasing leaf age might simply be attributed to metabolic differences among different leaves. However, in this experiment, the leaf NRA in leaf of different positions of *C. acuminata* seedlings differed to that of other reports. And the exact reasons are needed to be further characterized.

**Diurnal variation rhythm in leaf NRA**

From Fig.3, NRA increased rapidly in the morning, and reached the maximum, 0.74 $\mu mol \cdot h^{-1} \cdot g^{-1}$ (FW) at about midday (at 12:30 or so). After that, it presented a continu-

ous decrease, and remained at a relatively high level after 15:30 hour. The difference between the maximum and minimum leaf NRA was 0.6 $\mu mol \cdot h^{-1} \cdot g^{-1}$ (FW), and the fluctuation range of NRA level was slighter in the afternoon than in the morning.

Diurnal fluctuation of NRA has been observed in a number of plant species (Zhou *et al.* 1998), but no consistent conclusion can be obtained to explain the diurnal fluctuation rhythm in NRA up to now. Similar to spinach, leaf NRA in maize increased rapidly to the peak within 2 h, and then began to decline, but generally remained relatively high throughout the day (Huber *et al.* 1994). The similar diurnal regulation of NRA was also observed in the leaves of grapevine and tobacco (Hunter *et al.* 1997; Geiger *et al.* 1998). Huber *et al.* (1994) suggested that metabolites produced by photosynthetic metabolism and light influenced the diurnal variation in NRA levels in maize leaves. However, in cotton, leaf NRA increased rapidly in the first hour of illumination, reached a relatively stable level, and remained at that level for the next 6 h. After that, NRA decreases gradually to the least, at the end of the light period. Aslam *et al.* (2001) suggested that the availability of nitrate at the site of reduction, rather than the limit of energy supply produced by photosynthesis primarily affected the diurnal rhythm of NRA in cotton. Wen *et al.* (1999) found that leaf NRA in soybean increased with the increment of light intensity, reached a peak at 12:00-15:00, and then declined gradually with the decrease of light intensity in the afternoon. He suggested that photosynthesis may have an effect on NRA, and the photosynthate may provide reductant for NR or $NO_3^-$ reduction reaction. The diurnal variation rhythm in NRA in leaves of *C. acuminata* seedlings was similar to that observed in soybean.

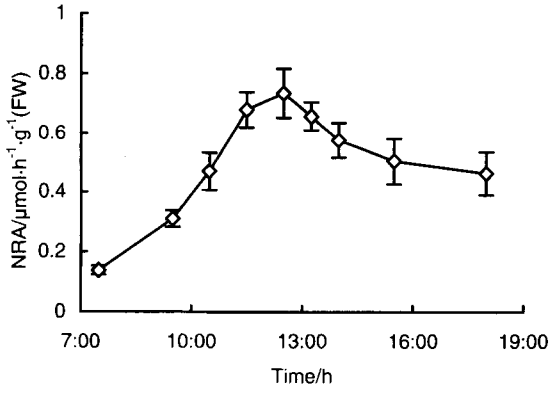


**Fig. 3 Diurnal variation rhythm of NRA in leaves of *C. acuminata* seedlings**

### Relationship between leaf NRA and leaf characteristics

No obvious correlation was displayed between leaf NRA and leaf area (Fig. 4). LMA is also an important index. No clear correlation was also found between leaf NRA and LMA (Fig. 5).

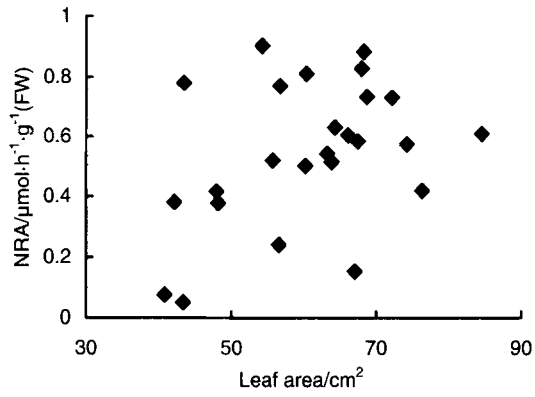


**Fig. 4 Relationship between leaf area and NRA in *C. acuminata* seedlings**

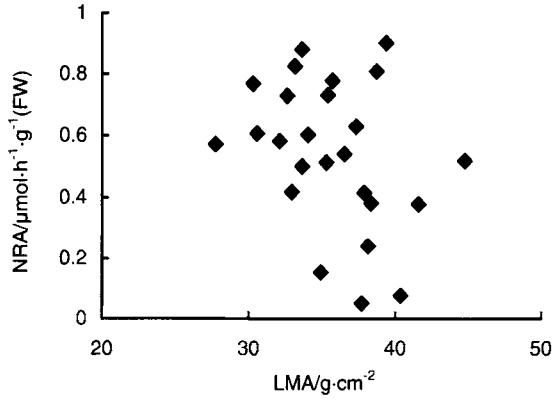


**Fig. 5 Relationship between LMA and NRA in *C. acuminata* seedlings**

## Conclusions

NRA in leaves of *C. acuminata* was much higher than that in the other organs. NRA changed distinctly in leaves of different positions, and relatively higher NRA was observed in leaf 4 to leaf 6. The peak NRA in leaves occurred at about midday. Leaf NRA was not correlated to leaf area and LMA.

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